APPENDIX 3

Please cancel claims 23 and 121-131 without prejudice; please amend the claims as follows; and please add new claims 167-169 as follows:

1. (Amended) A method for obtaining a bioactivity or a biomolecule of interest, comprising:

a) screening a library of clones generated from nucleic acids obtained directly from a mixed population of cells, for a specified bioactivity or biomolecule;

b) [variegating] mutating a nucleic acid sequence contained in a clone from the library having the specified bioactivity or biomolecule; and

c) comparing the bioactivity or biomolecule from b) with the specified bioactivity or biomolecule wherein a difference in the bioactivity or biomolecule is indicative of an effect of sequence [variegation] mutation, thereby providing the bioactivity or biomolecule of interest.

6. (Amended) The method of claim 4, wherein the detectable signal is [optical] fluorescence.

7. (Amended) The method of claim 5, wherein the fluorogenic substrate is umbelliferone or a derivative [or analogue] thereof, resorufin or a derivative [or analogue thereof], fluorescein or a derivative [or analogue] thereof, or rohodamine or a derivative [or analogue] thereof.

12. (Amended) The method of claim 2, further comprising comparing the [variegated] mutated nucleic acid sequence of interest to the [non-variegated] non-mutated nucleic acid sequence [of (c),] [thereby] to identify[ing] the nucleotide sequence [variegation] mutation.

22. (Amended) The method of claim 17, wherein the screening comprises contacting a clone with a substrate [labeled with a detectable molecule] wherein interaction of the substrate with the bioactivity or biomolecule contained in the clone produces a detectable signal.

27. (Amended) The method of claim 1, further comprising, prior to [(d)] (a), obtaining nucleic acids from the clone containing the specified bioactivity or biomolecule.

30. (Amended) The method of claim 1, wherein the nucleic acid sequence is [variegated] mutated by a method selected from the group consisting of error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, *in vivo* mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, ligation reassembly, GSSM and any combination thereof.

31. (Amended) The method of claim 1, wherein nucleic acid sequence is [variegated] mutated by error-prone PCR.

32. (Amended) The method of claim 1, wherein the nucleic acid sequence is [variegated] mutated by shuffling.

33. (Amended) The method of claim 1, wherein the nucleic acid sequence is [variegated] mutated by oligonucleotide-directed mutagenesis.

34. (Amended) The method of claim 1, wherein the nucleic acid sequence is [variegated] mutated by assembly PCR.

35. (Amended) The method of claim 1, wherein the nucleic acid sequence is [variegated] mutated by sexual PCR mutagenesis.

36. (Amended) The method of claim 1, wherein the nucleic acid sequence is [variegated] mutated by *in vivo* mutagenesis.

37. (Amended) The method of claim 1, wherein the nucleic acid sequence is [variegated] mutated by cassette mutagenesis.

38. (Amended) The method of claim 1, wherein the nucleic acid sequence is [variegated] mutated by recursive ensemble mutagenesis.

39. (Amended) The method of claim 1, wherein the nucleic acid sequence is [variegated] mutated by exponential ensemble mutagenesis.

40. (Amended) The method of claim 1, wherein the nucleic acid sequence is [variegated] mutated by site-specific mutagenesis.

41. (Amended) The method of claim 1, comprising screening the clone of [(c)] (b) for a further specified protein or enzymatic activity[,] prior to [variegating] mutating the nucleic acids.

48. (Amended) The method of claim 1, wherein the library is screened by contacting [or encapsulating] a clone of the library with a [bioactive] substrate, wherein a bioactivity or biomolecule produced by the clone is detectable by a difference in the substrate prior to contacting with the clone as compared to after contacting.

52. (Amended) A method for identifying a bioactivity or a biomolecule of interest, comprising:

a) screening a library of clones generated from pooled nucleic acids obtained directly from a plurality of isolates for a specified bioactivity or biomolecule; and

b) identifying a clone which contains the specified bioactivity or biomolecule.

53. (Amended) A method for identifying a bioactivity or a biomolecule of interest, comprising:

a) screening a library of clones generated from pooled nucleic acids obtained directly from a plurality of isolates for a specified bioactivity or biomolecule;

b) [variegating] mutating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

c) comparing the bioactivity or biomolecule from b) with the specified bioactivity or biomolecule wherein a difference in the bioactivity or biomolecule is indicative of an effect of introducing at least one sequence [variegation] mutation, thereby providing the bioactivity or biomolecule of interest.

54. (Amended) A method for identifying a bioactivity or a biomolecule of interest, comprising:

a) screening a library of clones generated from pooling individual gene libraries generated from the nucleic acids obtained directly from each of a plurality of isolates for a specified bioactivity or biomolecule; and

b) identifying a clone which contains the specified bioactivity or biomolecule.

55. (Amended) A method for identifying a bioactivity or a biomolecule of interest, comprising:

a) screening a library for a specified bioactivity or biomolecule wherein the library is generated from pooling individual gene libraries generated from the nucleic acids obtained directly from each of a plurality of isolates;

b) [variegating] mutating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

c) comparing the bioactivity or biomolecule from c) with the specified bioactivity or biomolecule wherein a difference in the bioactivity or biomolecule is indicative of an effect of introducing at least one sequence [variegation] mutation, thereby providing the bioactivity or biomolecule of interest.

57. (Amended) A method of identifying a bioactivity or biomolecule of interest, comprising:

a) screening a library of clones generated from nucleic acids obtained directly from an enriched population of organisms for a specified bioactivity or biomolecule;

b) [variegating] mutating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

c) comparing the bioactivity or biomolecule from b) with the specified bioactivity or biomolecule wherein a difference in the bioactivity or biomolecule is indicative of an effect of introducing at least one sequence [variegation] mutation, thereby providing the bioactivity or biomolecule of interest.

58. (Amended) A method for identifying a bioactivity or a biomolecule of interest, comprising:

(a) incubating nucleic acids obtained directly from a mixed population of organisms with at least one oligonucleotide probe comprising a detectable molecule and at least a portion of a nucleic acid sequence encoding a molecule of interest under such conditions and such time to allow interaction of complementary sequences;

(b) identifying nucleic acid sequences having a complement to the oligonucleotide probe using an analyzer that detects the detectable molecule;

(c) generating a library from the identified nucleic acid sequences;

(d) screening the library for a specified bioactivity or biomolecule;

(e) [variegating] mutagenizing a nucleic acid sequence contained in a clone from the library having the specified bioactivity or biomolecule; and

(f) comparing the bioactivity or biomolecule product from (e) with the specified bioactivity or biomolecule wherein a difference in the bioactivity or biomolecule is indicative of an effect of introducing at least one sequence [variation] mutation, thereby providing the bioactivity or biomolecule of interest.

59. (Amended) A method for identifying a bioactivity or a biomolecule of interest, comprising:

(a) co-encapsulating in a microenvironment nucleic acids obtained directly from a mixed population of organisms, with at least one oligonucleotide probe comprising a detectable molecule and at least a portion of a nucleic acid sequence encoding a molecule of interest under such conditions and for such time as to allow interaction of complementary sequences;

(b) identifying encapsulated nucleic acids containing a complement to the oligonucleotide probe encoding the molecule of interest by separating the encapsulated nucleic acids with an analyzer that detects the detectable molecule;

(c) generating a library from the separated encapsulated nucleic acids;

(d) screening the library for a specified bioactivity or biomolecule;

(e) [variegating] mutating a nucleic acid sequence contained in a clone from the library having the specified bioactivity or biomolecule; and

(f) comparing the bioactivity or biomolecule product from (e) with the specified bioactivity or biomolecule wherein a difference in the bioactivity or biomolecule is indicative of an effect of introducing at least one sequence [variation] mutation, thereby providing the bioactivity or biomolecule of interest.

60. (Amended) A method for identifying a bioactivity or a biomolecule of interest, comprising:

(a) co-encapsulating in a microenvironment nucleic acids obtained directly from an isolate of a mixed population of organisms, with at least one oligonucleotide probe comprising a detectable marker and at least a portion of a polynucleotide sequence encoding a molecule having a bioactivity of interest under such conditions and for such time as to allow interaction of complementary sequences;

(b) identifying encapsulated nucleic acids containing a complement to the oligonucleotide probe encoding the molecule or interest by separating the encapsulated nucleic acids with an analyzer that detects the detectable marker;

(c) generating a library from the separated encapsulated nucleic acids;

(d) screening the library for a specified bioactivity or biomolecule;

(e) [variegating] mutating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

(f) comparing the bioactivity or biomolecule product from (e) with the specified bioactivity or biomolecule wherein a difference in the bioactivity or biomolecule is indicative of an effect of introducing at least one sequence [variation] mutation, thereby providing the bioactivity or biomolecule of interest.

61. (Amended) A method for obtaining a bioactivity or a biomolecule of interest, comprising:

(a) co-encapsulating in a microenvironment nucleic acids obtained directly from one or more isolates of a mixed population of organisms, with at least one oligonucleotide probe comprising a detectable marker and at least a portion of a polynucleotide sequence encoding a molecule having a bioactivity of interest under such conditions and for such time as to allow interaction of complementary sequences;

(b) identifying encapsulated nucleic acids containing a complement to the oligonucleotide probe encoding the molecule of interest by separating the encapsulated nucleic acids with an analyzer that detects the detectable marker;

(c) generating a library from the separated encapsulated nucleic acids;

(d) screening the library for a specified bioactivity or biomoleeule;

(e) [variegating] mutating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

(f) comparing the bioactivity or biomolecule product from (e) with the specified bioactivity or biomolecule wherein a difference in the bioactivity or biomolecule is indicative of an effect of introducing at least one sequence [variation] mutation, thereby providing the bioactivity or biomolecule of interest.

62. (Amended) A method for identifying a bioactivity or a biomolecule of interest, comprising:

a) co-encapsulating in a microenvironment nucleic acids obtained directly from a mixture of isolates of a mixed population of organisms, with at least one oligonucleotide probe comprising a detectable marker and at least a portion of a polynucleotide sequence encoding a molecule having a bioactivity of interest under such conditions and for such time as to allow interaction of complementary sequences;

b) identifying encapsulated nucleic acids containing a complement to the oligonucleotide probe encoding the molecule of interest by separating the encapsulated nucleic acids with an analyzer that detects the detectable marker;

c) generating a library from the separated encapsulated nucleic acids;

d) screening the library for a specified bioactivity or biomolecule;

e) [variegating] mutating the a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

f) comparing the bioactivity or biomolecule product from (e) with the specified bioactivity or biomolecule wherein a difference in the bioactivity or biomolecule is indicative of an effect of introducing at least one sequence [variation] mutation, thereby providing the bioactivity or biomolecule of interest.

63. (Amended) A method for obtaining a bioactivity or a biomolecule of interest, comprising:

a) screening a library of clones generated from nucleic acids obtained directly from a mixed population of cells, for a specified bioactivity or biomolecule;

b) [variegating] mutating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

c) screening the [variegated] mutated nucleic acid sequence to obtain the biomolecule or the bioactivity of interest.

68. (Amended) The method of claim 66, wherein the detectable signal is [optical] fluorescence.

69. (Amended) The method of claim 67, wherein the fluorogenic substrate is umbelliferone or a derivative [or analogue] thereof, resorufin or a derivative [or analogue thereof], fluorescein or a derivative [or analogue] thereof, or rohodamine or a derivative [or analogue] thereof.

72. (Amended) The method of claim [64] 63, wherein the screening is by PCR amplification of a nucleic acid sequence of interest using primers substantially complementary to the sequence of interest or sequences flanking a nucleic acid of interest and having a detectable molecule.

73. (Amended) The method of claim [64] 63, wherein the screening is by hybridization of an oligonucleotide substantially complementary to a nucleic acid sequence of interest and having a detectable molecule.

82. (Amended) The method of claim [81] 80, wherein the extremeophiles are selected from the group consisting of hyperthermophiles, psychrophiles, halophiles, psychrotrophs, alkalophiles, and acidophiles.

87. (Amended) The method of claim 63, wherein the nucleic acid sequence is [variegated] mutated by a method selected from the group consisting of error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR

mutagenesis, *in vivo* mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, ligation reassembly, GSSM and any combination thereof.

88. (Amended) The method of claim 63, wherein the nucleic acid sequence is [variegated] mutated by error-prone PCR.

89. (Amended) The method of claim 63, wherein the nucleic acid sequence is [variegated] mutated by shuffling.

90. (Amended) The method of claim 63, wherein the nucleic acid sequence is [variegated] mutated by oligonucleotide-directed mutagenesis.

91. (Amended) The method of claim 63, wherein the nucleic acid sequence is [variegated] mutated by assembly PCR.

92. (Amended) The method of claim 63, wherein the nucleic acid sequence is [variegated] mutated by sexual PCR mutagenesis.

93. (Amended) The method of claim 63, wherein the nucleic acid sequence is [variegated] mutated by *in vivo* mutagenesis.

94. (Amended) The method of claim 63, wherein the nucleic acid sequence is [variegated] mutated by cassette mutagenesis.

95. (Amended) The method of claim 63, wherein the nucleic acid sequence is [variegated] mutated by recursive ensemble mutagenesis.

96. (Amended) The method of claim 63, wherein the nucleic acid sequence is [variegated] mutated by exponential ensemble mutagenesis.

97. (Amended) The method of claim 63, wherein the nucleic acid sequence is [variegated] mutated by site-specific mutagenesis.

98. (Amended) The method of claim 63, comprising screening the clone of (c) for a further specified protein or enzymatic activity, prior to [variegating] mutating the nucleic acids.

105. (Amended) The method of claim 63, wherein the library is screened by contacting [or encapsulating] a clone of the library with [bioactive] a substrate, wherein a bioactivity or biomolecule produced by the clone is detectable by a difference in the substrate prior to contacting with the clone as compared to after contacting.

107. (Amended) The method of claim 63, wherein the [bioactivity or] biomolecule is a gene cluster or fragment thereof.

108. (Amended) The method of claim 63, wherein the [bioactivity or] biomolecule is a polypeptide in a metabolic pathway.

109. (Amended) A method for identifying a bioactivity or a biomolecule of interest, comprising:

a) screening a library for a specified bioactivity or biomolecule wherein the library is generated from pooling individual gene libraries generated from the nucleic acids obtained directly from each of a plurality of isolates;

b) [variegating] mutating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

c) screening the [variegated] mutated nucleic acid sequence to obtain the biomolecule or the bioactivity of interest.

110. (Amended) A method of identifying a bioactivity or biomolecule of interest, comprising:

a) screening a library of clones generated from nucleic acids obtained directly from an enriched population of organisms for a specified bioactivity or biomolecule;

b) [variegating] mutating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

c) screening the [variegated] mutated nucleic acid sequence to obtain the biomolecule or the bioactivity of interest.

111. (Amended) A method for identifying a bioactivity or a biomolecule of interest, comprising:

a) incubating nucleic acids obtained directly from a mixed population of organisms with at least one oligonucleotide probe comprising a detectable molecule and at least a portion of a nucleic acid sequence encoding a molecule of interest under such conditions and such time to allow interaction of complementary sequences;

b) identifying nucleic acid sequences having a complement to the oligonucleotide probe using an analyzer that detects the detectable molecule;

c) generating a library from the identified nucleic acid sequences;

d) screening the library for a specified bioactivity or biomolecule;

e) [variegating] mutating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

f) screening the [variegated] mutated nucleic acid sequence to obtain the biomolecule or the bioactivity of interest.

112. (Amended) A method for identifying a bioactivity or a biomolecule of interest, comprising:

a) co-encapsulating in a microenvironment nucleic acids obtained directly from a mixed population of organisms, with at least one oligonucleotide probe

comprising a detectable molecule and at least a portion of a nucleic acid sequence encoding a molecule of interest under such conditions and for such time as to allow interaction of complementary sequences;

b) identifying encapsulated nucleic acids containing a complement to the oligonucleotide probe encoding the molecule of interest by separating the encapsulated nucleic acids with an analyzer that detects the detectable molecule;

c) generating a library from the separated encapsulated nucleic acids;

d) screening the library for a specified bioactivity or biomolecule;

e) [variegating] mutating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

f) screening the [variegated] mutated nucleic acid sequence to obtain the biomolecule or the bioactivity of interest.

113. (Amended) A method for identifying a bioactivity or a biomolecule of interest, comprising:

a) co-encapsulating in a microenvironment nucleic acids obtained directly from an isolate of a mixed population of organisms, with at least one oligonucleotide probe comprising a detectable marker and at least a portion of a polynucleotide sequence encoding a molecule having a bioactivity of interest under such conditions and for such time as to allow interaction of complementary sequences;

b) identifying encapsulated nucleic acids containing a complement to the oligonucleotide probe encoding the molecule of interest by separating the encapsulated nucleic acids with an analyzer that detects the detectable marker;

c) generating a library from the separated encapsulated nucleic acids;

d) screening the library for a specified bioactivity or biomolecule;

e) [variegating] mutating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

f) screening the [variegated] mutated nucleic acid sequence to obtain the biomolecule or the bioactivity of interest.

114. (Amended) A method for obtaining a bioactivity or a biomolecule of interest. comprising:

a) co-encapsulating in a microenvironment nucleic acids obtained directly from one or more isolates of a mixed population. of organisms, with at least one oligonucleotide probe comprising a detectable marker and at least a portion of a polynucleotide sequence encoding a molecule having a bioactivity of interest under such conditions and for such time as to allow interaction of complementary sequences;

b) identifying encapsulated nucleic acids containing a complement to the oligonucleotide probe encoding the molecule of interest by separating the encapsulated nucleic acids with an analyzer that detects the detectable marker;

c) generating a library from the separated encapsulated nucleic acids;

d) screening the library for a specified bioactivity or biomolecule;

e) [variegating] mutating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

f) screening the [variegated] mutated nucleic acid sequence to obtain the biomolecule or the bioactivity of interest.

115. (Amended) A method for identifying a bioactivity or a biomolecule of interest, comprising:

a) co-encapsulating in a microenvironment nucleic acids obtained directly from a mixture of isolates of a mixed population of organisms, with at least one oligonucleotide probe comprising a detectable marker and at least a portion of a polynucleotide sequence encoding a molecule having a bioactivity of interest under such conditions and for such time as to allow interaction of complementary sequences;

b) identifying encapsulated nucleic acids containing a complement to the oligonucleotide probe encoding the molecule of interest by separating the encapsulated nucleic acids with an analyzer that detects the detectable marker;

c) generating a library from the separated encapsulated nucleic acids;

d) screening the library for a specified bioactivity or biomolecule;

e) [variegating] mutating the a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

f) screening the [variegated] mutated nucleic acid sequence to obtain the biomolecule or the bioactivity of interest.

119. (Amended) The method of claim 116, further comprising the step of:

expressing the mutagenized molecule [of step (b)] to create a bioactivity or biomolecule containing a mutation.

135. (Amended) The method of claim 133, wherein the operon produces a [molecule selected from a] polyketide synthase[, a polyketides, an anti-cancer agent, and an imunosuppressant].

137. (Amended) The method of claim 116, wherein the DNA molecules are inserted into a vector prior to [step a)] said creating a DNA library.

Please enter the following new claims:

167. (New) The method of claim 133, wherein the operon produces a polyketide.

168. (New) The method of claim 133, wherein the operon produces an anti-cancer agent.

169. (New) The method of claim 133, wherein the operon produces an immunosuppressant.